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Effects of *Paullinia cupana* extract on lamotrigine pharmacokinetics in rats: A herb-drug interaction on the gastrointestinal tract with potential clinical impact

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ABSTRACT

Paullinia cupana-containing preparations are being consumed worldwide for weight reduction. As obesity and epilepsy are common comorbidities and lamotrigine (LTG) is a broad-spectrum antiepileptic drug, it is likely to find epilepsy patients taking *P. cupana* and LTG simultaneously. Thus, this work aimed to investigate the potential interaction between *P. cupana* extract and LTG in rats. In a study, rats were orally co-administered with a single-dose of *P. cupana* extract (821 mg/kg) and LTG (10 mg/kg). In another study, rats were orally pre-treated for 14 days with *P. cupana* extract (821 mg/kg/day) receiving LTG (10 mg/kg, p.o.) on the 15th day. Rats of the respective control groups received the corresponding volume of the extract vehicle. LTG concentrations were determined at several post-dose time-points and submitted to a non-compartmental pharmacokinetic analysis. The co-administration of *P. cupana* and LTG induced a significant reduction of LTG C_{max} and $AUC_{0\text{-}24}$ and prolonged the mean residence time. However, no significant effects were observed on LTG pharmacokinetics following a 14-day pre-treatment period with the extract. In this study changes in the body weight of rats and in some biochemical parameters were also evaluated. Overall, the results revealed a pharmacokinetic-based herb-drug interaction between *P. cupana* extract and LTG, mainly after their co-administration.

1. Introduction

Paullinia cupana, also known as Guarana, is a species that belongs to the *Sapindaceae* family and it is being consumed worldwide in herbal supplements and stimulating drinks (Portella et al., 2013). This native Amazonian plant has been described as having stimulant effects and other medicinal properties (Schimpl et al., 2013), mainly due to the presence of caffeine (2–8%) in the seeds of its fruits. Other methylxanthines, like theophylline and theobromine, are also found in small amounts (< 0.3%) in the seeds, bark, flowers and leaves of *P. cupana* (Ashihara et al., 2008; Schimpl et al., 2013). Among several species of plants that produce caffeine, *P. cupana* has the higher natural content of this compound when compared to coffee (*Coffea arabica*), tea (*Camellia sinensis*) and yerba mate (*Ilex paraguariensis*) (Ashihara and Crozier, 2001; Ashihara et al., 2008). In fact, depending on how the extracts are prepared, *P. cupana* extracts may contain caffeine in an amount four times higher than that found in coffee beans (Moustakas et al., 2015). Other constituents that can be found in *P. cupana* seeds are polysaccharides, polyphenols (e.g. catechins, epicatechins and tannins), lipids, saponins, proteins, choline and pigments (Schimpl et al., 2013).

P. cupana has a well-established medicinal use for symptoms of fatigue and feeling of weakness (EMA, 2013). However, several other pharmacological effects have been related to *P. cupana* consumption, including antiplatelet aggregation, cardioprotective and chemopreventive effects, and also antioxidant, antidepressant, antimicrobial and anti-obesity properties (Hamerski et al., 2013). Some studies have demonstrated that *P. cupana*-containing products improve lipid metabolism, promote weight loss and increase the basal energy expenditure,

Abbreviations: AED, antiepileptic drug; AUC, area under the concentration-time curve; $AUC_{0\text{-}24}$, AUC from time zero to 24 h; $AUC_{0\text{-}t}$, AUC from time zero to the last measurable concentration; $AUC_{0\text{-}\infty}$, AUC from time zero to infinite; C_{max}, peak concentration; HPLC-DAD, high-performance liquid chromatography–diode array detection; IS, internal standard; k_{el}, apparent elimination rate constant; LTG, lamotrigine; MEPS, microextraction by packed sorbent; MRT, mean residence time; p.o., per os; $t_{1/2el}$, apparent terminal elimination half-life; t_{max}, time to reach peak concentration

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acting as thermogenics or metabolic stimulants (Glade, 2010; Hamerski et al., 2013; Portella et al., 2013). Indeed, caffeine increases the excitability of adenosine-sensitive sympathetic nervous system, stimulating fat lipolysis (Glade, 2010).

Overweight and obesity are widely recognized as modifiers of therapeutic response and prognosis of several chronic health conditions. More specifically, obesity has been commonly reported as a comorbid condition of epilepsy, with a high prevalence in children and adults (Arya et al., 2016; Janousek et al., 2013). Recent studies have focused on the association between overweight or obesity and epilepsy. For instance, Ladino et al. (2014) found that 72% of adult patients with epilepsy present overweight, obesity or even morbid obesity, corresponding respectively to 34%, 25% and 13%. Another study referred to that 55.2% of patients with epilepsy were overweight or obese (Janousek et al., 2013). There is also evidence that obesity is more common in patients with refractory epilepsy and in those treated in polytherapy regimens (Baxendale et al., 2015; Chukwu et al., 2014; Janousek et al., 2013). Despite the limited data supporting the role of obesity in seizure severity, obesity may play a central role in the worsening of this neurological disorder (Hafizi et al., 2017).

Taking into account that the use of herbal dietary supplements has increased worldwide at an unprecedented rate, and given the growing prevalence of obesity among patients with epilepsy, it is expected an increasing consumption of herbal weight loss medicines by this patient subpopulation over the coming years. Moreover, bearing in mind that some constituents of plant extracts have been identified as substrates, inducers and/or inhibitors of transporters and/or enzymes responsible for antiepileptic drugs (AEDs) biodisposition (Oga et al., 2015; Roe et al., 2016; Tarirai et al., 2010; Wu et al., 2015), it is important not to neglect the potential risks associated with the combined use of herbal medicinal products and AEDs, which may compromise the control of seizures and even increase the risk of adverse drug reactions.

As lamotrigine (LTG) is an AED extensively used in the clinical practice, particularly due to its broad spectrum of efficacy in several types of epileptic disorders (Patsalos, 2013), and considering its narrow therapeutic range (3–15 μg/mL) (Patsalos et al., 2017) and its pharmacokinetics variability and propensity to interact with other drugs (Patsalos, 2013), it is fully justified to investigate the effects of *P. cupana* extract on the pharmacokinetics of LTG. In fact, up to date, to the best of our knowledge, no study was previously conducted to evaluate the potential of interaction between *P. cupana* and LTG. Therefore, this work was planned to investigate whether a commercial standardized *P. cupana* extract may influence the absorption and biodisposition of LTG in rats after their oral co-administration and following a 14-day *P. cupana* pre-treatment period. In addition, the impact of the repeated treatment with *P. cupana* extract on the body weight of rats and in some relevant biochemical parameters was also evaluated.

2. Materials and methods

2.1. Herbal extract, drugs and materials

P. cupana extract from seeds, containing 12% of caffeine, was purchased from Bio Serae Laboratories (Bram, France) and the corresponding certificate of analysis was received and preserved. LTG dispersible tablets (Lamictal® 25 mg, GSK), chloramphenicol (Sigma–Aldrich, St Louis, USA), used as internal standard (IS), pentobarbital (Eutasil®, 200 mg/ml, Ceva Saúde Animal), sodium chloride 0.9% solution (Labesfal, Portugal), heparin sodium 5000 I.U./mL (B. Braun Medical, Portugal), polyurethane cannula (Introcan® Certo IV indwelling cannula 22G; 0.9 × 2.5 mm; B. Braun Melsungen AG, Germany), disposable cholesterol and triglycerides test strips (Accutrend®, Roche, Germany) and disposable blood glucose test strips (Freestyle Lite, Abbott®) were commercially acquired.

2.2. Animals

Thirty-four healthy adult male Wistar rats (247 ± 14 g) were obtained from local certified animal facilities (Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal) and housed at 12 h light/dark cycle under controlled environmental conditions (temperature 20 ± 2 °C; relative humidity 55 ± 5%). The animals were allowed free access to a standard rodent diet and water *ad libitum*.

The experimental procedures were approved by the Portuguese National Authority for Animal Health, Phytosanitation and Food Safety (DGAV – Direção Geral de Alimentação e Veterinária) and all the animal experiments were conducted in accordance with the European Directive (2010/63/EU) for animal experiments.

2.3. Preparation of herbal extract and lamotrigine solutions

P. cupana extract solution was daily prepared by dissolving the powdered extract in distilled water. The dose of *P. cupana* administrated to each animal was 821 mg/kg (p.o.), using an administration volume of 10 mL/kg of rat body weight. The selected dose was defined taking into account the human dose recommendation from the extract supplier, which was converted to rat species following a Food and Drug Administration (FDA) Guidance for Industry, which refers to the conversion of animal doses to human equivalent doses based on body surface area (FDA, 2005). Furthermore, a 10-fold potentiating interaction factor was employed to avoid false negative results.

LTG dispersible tablets were dissolved in a proper volume of distilled water to obtain the LTG solution for rat administration. A LTG dose of 10 mg/kg (p.o.) was administrated taking into consideration an administration volume of 4 mL/kg of rat body weight. LTG dose was selected according to the previous in-house group experience in rat studies, and taking also into account that with this dose, saturation phenomena in the processes of drug absorption and/or elimination are not probable to occur (Avula and Veesam, 2014; Ventura et al., 2016; Yamashita et al., 1997).

2.4. Systemic pharmacokinetic studies

Twenty-four rats were randomly distributed in four groups, each one containing six animals ($n = 6$). These studies were designed to investigate the effects of *P. cupana* extract on the bioavailability and plasmatic kinetics of LTG in two independent experimental assays. In the first pharmacokinetic study, rats of the experimental group were concomitantly treated with a single-oral dose of *P. cupana* extract (821 mg/kg, p.o.) and LTG (10 mg/kg, p.o.). In the second study, rats of the experimental group were orally pre-treated during 14 days with *P. cupana extract* (821 mg/kg/day, p.o.) followed by a single dose of LTG (10 mg/kg, p.o.) administrated on the 15th day. A 14-day period of time was considered for the repeated administration of the *P. cupana* extract based on available scientific literature (ICH, 2009; Ma and Ma, 2016), in which it is described that repeated administration studies should be conducted during at least 14 days. Rats of the control groups received the corresponding volume of the vehicle of the herbal extract (i.e. water) and were similarly treated with LTG.

In each study, on the night before LTG administration, each animal was anaesthetized for insertion of an Introcan® Certo IV indwelling cannula (22G; 0.9 × 2.5 mm) in a lateral tail vein for the subsequent serial blood sampling. Anesthesia was induced with pentobarbital (60 mg/kg) administered intraperitoneally. The rats fully recovered from anesthesia and were fasted before LTG administration, but they were maintained with free access to water. To avoid the food effect on LTG absorption and biodisposition, the fasting period was maintained until 4 h after drug administration.

LTG and *P. cupana extract* (or vehicle, in the control groups) were orally administrated by gavage during the morning in each study. After LTG administration, blood sampling was performed at 0.5, 1, 2, 4, 6, 8,

12, 24, 48, 72 and 96 h post-dose. Blood samples of approximately 0.3 mL were collected into EDTA tubes through the cannula inserted in the rat tail vein and then centrifuged at 4000 rpm for 10 min (4 °C) to separate the plasma which was stored at −20 ºC until analysis.

2.5. Plasma-to-brain biodistribution study

To further investigate the potential effects of *P. cupana* on the plasma-to-brain distribution of LTG an independent study was performed. In this study, ten rats were randomly distributed in two groups, each one containing five animals ($n = 5$). Each animal received by gavage a single oral dose of *P. cupana* extract (821 mg/kg, p.o.) or vehicle (in the control group) co-administrated with a single-oral dose of LTG (10 mg/kg, p.o.). Then, in order to measure the LTG concentrations achieved in plasma and brain at 6 h post-dose, rats were anaesthetized and sacrificed by decapitation. Blood samples were collected and centrifuged as previously described, and the resulting plasma was stored at −20 ºC until analysis. Brain tissue was quickly excised after exsanguination, weighed and homogenized in 0.1 M sodium phosphate buffer at pH 5.5 (4 mL per gram of tissue) using an Ultra-Turrax® tissue homogenizer. The brain tissue homogenates were centrifuged at 13,500 rpm for 10 min (4 °C) and the supernatants were collected and stored at −20 °C until use.

2.6. Liquid chromatography analysis

LTG concentrations in plasma and brain homogenate samples were determined using a microextraction by packed sorbent (MEPS) procedure combined with a high-performance liquid chromatography–diode array detection (HPLC-DAD) method previously developed and validated (Ventura et al., 2016). Briefly, to each aliquot (100 µL) of plasma or brain homogenate supernatant, spiked with 20 µL of the IS working solution (250 µg/mL), was added 400 µL of ice-cold acetonitrile (precipitating agent). The mixture was vortex-mixed for 30 s and centrifuged at 13,500 rpm for 10 min to precipitate proteins. The clear supernatant was collected and evaporated to dryness under a gentle nitrogen stream at 45 °C. The dry residue was reconstituted with 200 µL of 0.3% triethylamine-water solution (pH 6.5). Each reconstituted sample was extracted in three draw-eject cycles through the MEPS syringe, at a flow rate of 10 µL/s. The sorbent was then washed once with 200 µL ultra-pure water and, after that, LTG and IS were eluted with methanol (2 × 30 µL). This methanolic extract was diluted with 90 µL of ultra-pure water and 20 µL were injected into the chromatographic system. The lower limit of quantification was established at 0.1 µg/mL for LTG in plasma and in brain tissue homogenate.

2.7. Pharmacokinetic analysis

The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) of LTG were obtained directly from the experimental data. The remaining pharmacokinetic parameters were estimated from the individual plasma concentration-time profiles by non-compartmental pharmacokinetic analysis using WinNonlin version 5.2 (Pharsight Co, Mountain View, CA, USA). For each animal, the estimated pharmacokinetic parameters included the truncated area under the concentration-time curve (AUC) from time zero to 24 h ($AUC_{0\text{-}24}$), AUC from time zero to the last measurable concentration ($AUC_{0\text{-}t}$), which were calculated by the linear trapezoidal rule; and the AUC from time zero to infinite ($AUC_{0\text{-}\infty}$), which was determined from $AUC_{0\text{-}t} + (C_{last}/k_{el})$, where C_{last} is the quantifiable concentration at the time of the last measurable drug concentration (t_{last}) and k_{el} is the apparent elimination rate constant calculated by log-linear regression of the terminal segment of the concentration-time profile. The apparent terminal elimination half-life ($t_{1/2el}$) and the mean residence time (MRT) were also estimated. The drug concentrations below the lower limit of quantification of the assay were taken as zero for all calculations.

2.8. Effects of repeated-dose administration of P. cupana extract on biochemical parameters

To assess the effects of repeated treatment with *P. cupana* extract on biochemical parameters, the blood levels of glucose, total cholesterol and triglycerides were evaluated in all rats of the experimental (*P. cupana*) and control (vehicle) groups on the 14th day of the *P. cupana* pretreatment study and compared. The blood determination of these three biochemical parameters was performed using appropriate medical devices (Accutrend® Plus, Roche, for cholesterol and triglycerides analysis; and Freestyle Freedom Lite, Abbott®, for glucose analysis) and the corresponding disposable test strips.

2.9. Effects of repeated-dose administration of P. cupana extract on body weight

To evaluate the effects of *P. cupana* extract on rats' body weight over the 14 days of treatment, the body weight of the animals of both experimental (*P. cupana*) and control (vehicle) groups was determined in the first and last day (14th) of the *P. cupana* pretreatment study, and then compared.

2.10. Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM), except for t_{max}. As t_{max} is a categorical variable in the performed pharmacokinetic studies, which can only take values based on planned sampling schedule, t_{max} values were expressed as median and range. Non-parametric Mann-Whitney test was used to compare the t_{max} values from two different groups. The statistical comparisons of the other pharmacokinetic parameters, body weight and biochemical markers between two groups were performed using unpaired two-tailed Student's *t*-test; in addition, for comparisons of body weight changes within the same group a paired Student's *t*-test was employed. A difference was considered to be statistically significant for a *p*-value lower than 0.05 ($p < 0.05$).

3. Results

3.1. Effects of P. cupana extract on LTG pharmacokinetics after co-administration

The mean plasma concentration-time profiles ($n = 6$) of LTG obtained in rats after the simultaneous administration of a single-oral dose of *P. cupana* extract (821 mg/kg) or vehicle and the drug itself (10 mg/kg) are represented in Fig. 1, and the corresponding pharmacokinetic parameters estimated by applying non-compartmental analysis to each individual concentration-time profile are summarized in Table 1. From the observation of the mean plasma pharmacokinetic profiles (Fig. 1), it is evident the occurrence of important differences in the extent of systemic exposure to LTG, which was considerably reduced in the presence of *P. cupana* extract. A statistically significant decrease in LTG plasma concentrations was observed in the experimental group, between 0.5 h and 8 h, when compared to the control group ($p < 0.05$). The effect of *P. cupana* extract was found to be particularly marked on the LTG C_{max} and truncated $AUC_{0\text{-}24}$, which were reduced by 32.6% and 36.6%, respectively ($p < 0.05$) (Table 1). Nevertheless, only a slight reduction was observed in the extent of total systemic drug exposure (as assessed by $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$). Despite the statistically significant differences found in the extent of systemic drug exposure achieved up to 24 h post-dose, the time to reach the peak plasma concentration of LTG was similar in both experimental (*P. cupana*) and control (vehicle) groups. Specifically, as shown in Table 1, the median values for t_{max} were 4 h in both experimental and control groups, with mean values of 8 h and 5.58 h for *P. cupana* extract group and control group, respectively. Additionally, a statistically significant increase of the MRT value

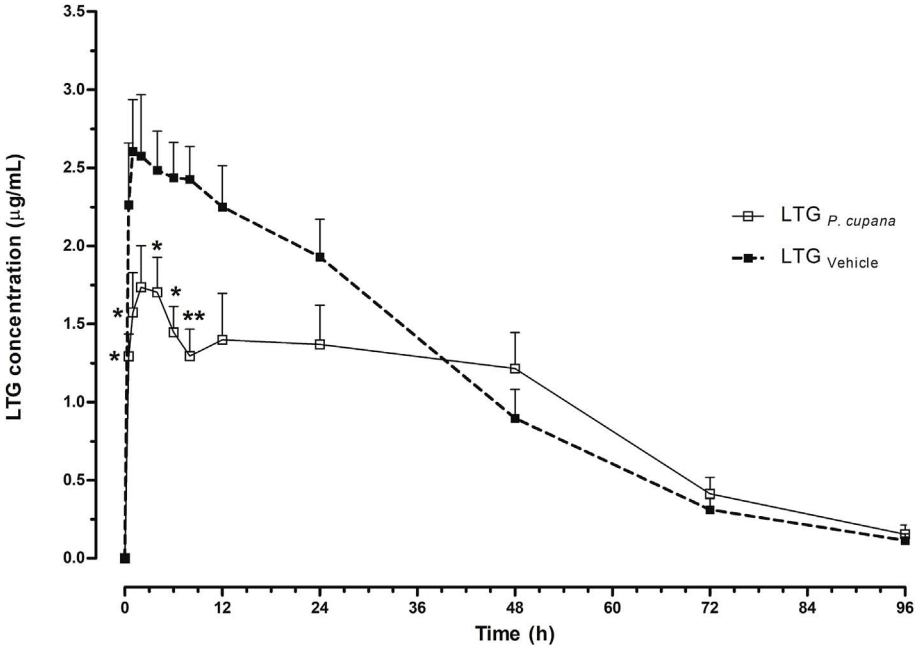

Fig. 1. Mean plasma concentration-time profiles of lamotrigine (LTG) obtained, over a period of 96 h, from rats co-administered with a single-dose of Paullinia cupana extract (821 mg/kg, p.o.) or vehicle of the extract (i.e. water) and LTG (10 mg/kg, p.o.) by oral gavage. Symbols represent the mean values ± standard error of the mean (SEM) of six determinations per time point (n = 6). *p < 0.05 and **p < 0.005 compared to control (vehicle).

Table 1
Pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of lamotrigine (LTG) obtained in rats after the co-administration with a single-dose of *Paullinia cupana* extract (821 mg/kg, p.o.) or vehicle of the extract (i.e. water) and LTG (10 mg/kg, p.o.) by oral gavage (n = 6). Data are presented as mean values ± standard error of the mean (SEM), except for t_{max} that is expressed as median values (range).

Parameter	Experimental Group LTG $_{P.\ cupana}$	Control Group LTG $_{vehicle}$
C_{max} (μg/mL)	2.022 ± 0.222 *	3.00 ± 0.284
t_{max} (h)	4.0 (2.0–24.0)	4.0 (0.5–12.0)
$AUC_{0\text{-}24}$ (μg.h/mL)	34.028 ± 4.229*	53.642 ± 4.990
$AUC_{0\text{-}t}$ (μg.h/mL)	90.383 ± 13.242	106.488 ± 11.692
$AUC_{0\text{-}\infty}$ (μg.h/mL)	96.842 ± 13.890	110.660 ± 12.103
k_{el} (1/h)	0.0386 ± 0.0030	0.0415 ± 0.0020
$t_{1/2el}$ (h)	18.5 ± 1.5	16.9 ± 0.8
MRT (h)	38.6 ± 3.1 *	29.1 ± 2.2

AUC, area under the concentration-time curve; $AUC_{0\text{-}24}$, AUC from time zero to 24 h; $AUC_{0\text{-}t}$, AUC from time zero to the last measurable concentration; $AUC_{0\text{-}\infty}$, AUC from time zero to infinite; C_{max}, peak concentration; k_{el}, apparent elimination rate constant; MRT, mean residence time; $t_{1/2el}$, apparent terminal elimination half-life; t_{max}, time to reach peak concentration. *p < 0.05, significantly different from the control (vehicle) group.

of LTG was observed in the experimental group when compared to the control group (p < 0.05). On the other hand, the mean values estimated for the elimination pharmacokinetic parameters (k_{el} and $t_{1/2el}$) of LTG were similar in both groups (*P. cupana* extract *versus* vehicle) (Table 1).

3.2. Effects of repeated-dose pretreatment with P. cupana extract on LTG pharmacokinetics

The mean plasma pharmacokinetic profiles of LTG following a single-oral administration of 10 mg/kg of the drug (at 15th day) to rats previously submitted to a 14-day treatment period with *P. cupana* extract (821 mg/kg/day) or vehicle are depicted in Fig. 2. In addition, the respective mean (or median) pharmacokinetic parameters are presented in Table 2. A similar pattern of the plasma concentration-time curves was observed in both experimental (*P. cupana*) and control (vehicle) groups, although slightly higher LTG concentrations were obtained in the experimental group over most of the study time, presenting statistically significant differences only at 72 h and 96 h post-dose (p < 0.05). C_{max} was slightly higher in the experimental group (16.9%) compared to the control group. The median LTG t_{max} was 9 h in the experimental group and 12 h in the control group, ranging the t_{max} values from 4 to 24 h (Table 2). Also worthy of note are the values estimated for the truncated $AUC_{0\text{-}24}$, which were very similar in both groups. Regarding the $AUC_{0\text{-}t}$ parameter no statistically significant differences (p > 0.05) were detected, but considering the mean values, there was a trend towards a higher systemic exposure (36.7%) in the group of rats treated with *P. cupana* extract. Otherwise, despite the 96-h sampling period established for this study, it was not possible to appropriately characterize the terminal elimination phase of the pharmacokinetic profile of LTG in some rats of the experimental group; thus, in this case, no reliable conclusions can be drawn by the comparison of the average values calculated for secondary pharmacokinetic parameters, which are highly influenced by the measurements in the terminal elimination phase of the concentration-time curve (k_{el}, $t_{1/2el}$, MRT and $AUC_{0\text{-}\infty}$).

3.3. Effects of P. cupana extract on the LTG plasma-to-brain biodistribution after co-administration

As LTG needs to cross the blood-brain barrier to achieve its biophase, and considering the pharmacokinetic herb-drug interaction evidenced systemically after the co-administration of *P. cupana* extract and LTG, this additional study was designed to evaluate the impact of such herb-drug interaction on the LTG plasma-to-brain biodistribution, employing the same dosing regimen (i.e. a single-oral dose of 10 mg/kg of LTG and 821 mg/kg of *P. cupana* extract). For this purpose, LTG concentrations were measured in plasma and brain tissue of rats sacrificed at 6 h post-dose. This time-point was selected because, among the serial sampling time-points defined in the systemic pharmacokinetic study described in section 3.1, the 6 h represent a post-dose time-point that is very close to the median t_{max} value estimated (6.5 h) considering together the t_{max} data (n = 12) of both groups (experimental and control groups). The results obtained are shown in Fig. 3. Analyzing and comparing the data obtained in this study, it is evident that statistically significant differences were found between plasma concentrations of LTG measured in the groups of rats that received *P. cupana* extract and vehicle (1.926 ± 0.226 μg/mL *versus* 3.683 ± 0.239 μg/mL, p < 0.005). On the other hand, although the mean concentrations of LTG achieved in brain tissue were lower in the experimental (*P. cupana*)

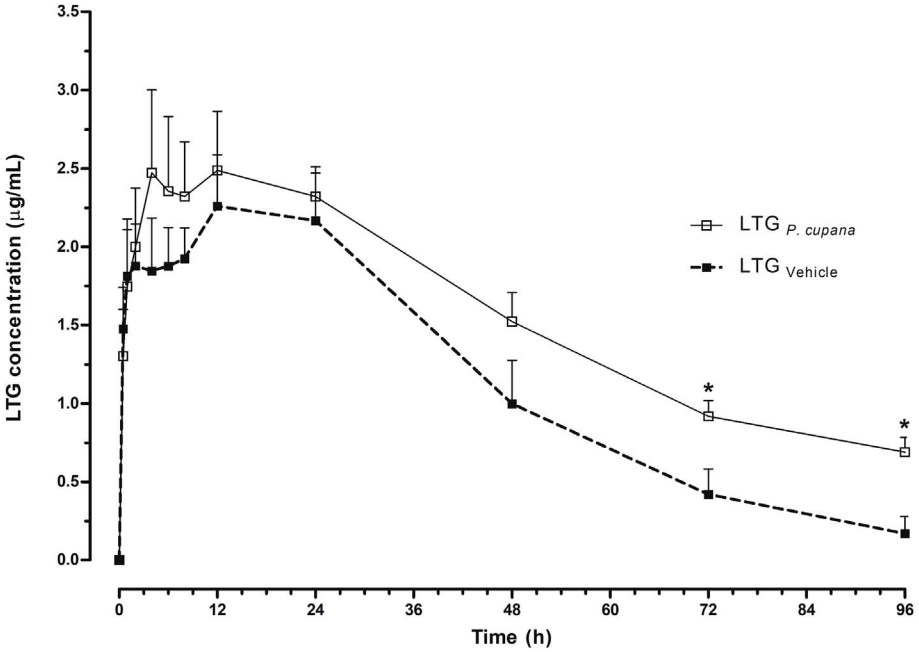

Fig. 2. Mean plasma concentration-time profiles of lamotrigine (LTG) obtained, over a period of 96 h, from rats submitted to a 14-day pre-treatment period with *Paullinia cupana* extract (821 mg/kg/day, p.o.) or vehicle of the extract (i.e. water), and treated on the 15th day with a single-dose of LTG (10 mg/kg, p.o.) by oral gavage. Symbols represent the mean values ± standard error of the mean (SEM) of six determinations per time point ($n = 6$). *$p < 0.05$ compared to control (vehicle).

Table 2
Pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of lamotrigine (LTG) obtained in rats submitted to a 14-day pre-treatment period with Paullinia cupana extract (821 mg/kg, p.o.) or vehicle of the extract (i.e. water), and treated on the 15th day with a single-dose of LTG (10 mg/kg, p.o.) by oral gavage (n = 6, unless otherwise noted). Data are presented as mean values ± standard error of the mean (SEM), except for tmax that is expressed as median values (range).

Parameter	Experimental Group LTG $_{P.\ cupana}$	Control Group LTG $_{vehicle}$
C_{max} (μg/mL)	3.165 ± 0.358	2.706 ± 0.237
t_{max} (h)	9.0 (4.0–24.0)	12.0 (4.0–24.0)
$AUC_{0\text{-}24}$ (μg.h/mL)	55.398 ± 6.973	49.195 ± 5.578
$AUC_{0\text{-}t}$ (μg.h/mL)	150.108 ± 11.737	109.770 ± 15.950
$AUC_{0\text{-}\infty}$ (μg.h/mL)	157.212 ± 26.779[a]	118.699 ± 18.679
k_{el} (1/h)	0.0229 ± 0.0041[a]	0.0419 ± 0.0050
$t_{1/2el}$ (h)	31.2 ± 5.6[a]	18.1 ± 2.5
MRT (h)	45.7 ± 10.3[a]	32.8 ± 5.2

AUC, area under the concentration-time curve; $AUC_{0\text{-}24}$, AUC from time zero to 24 h; $AUC_{0\text{-}t}$, AUC from time zero to the last measurable concentration; $AUC_{0\text{-}\infty}$, AUC from time zero to infinite; C_{max}, peak concentration; k_{el}, apparent elimination rate constant; MRT, mean residence time; $t_{1/2el}$, apparent terminal elimination half-life; t_{max}, time to reach peak concentration. [a] n = 2.

group (1.389 ± 0.217 μg/g) than in the control (vehicle) group (1.900 ± 0.256 μg/g), no statistically significant differences were found at this single point of sampling ($p > 0.05$).

3.4. Effects of repeated-dose administration of P. cupana extract on biochemical parameters

The blood levels of glucose, total cholesterol and triglycerides determined in rats treated repeatedly with *P. cupana* extract (experimental group) or vehicle (control group), over a period of 14 days, are shown in Fig. 4. Statistically significant differences were detected between experimental and control groups for glucose ($p < 0.005$) and triglycerides ($p < 0.05$) blood levels. The mean glucose levels measured in the rats of experimental (*P. cupana*) group were higher than those found in rats of control (vehicle) group, which were 74.2 ± 2.8 mg/dL and 56.0 ± 2.0 mg/dL, respectively. On the contrary, the mean triglyceride levels determined in the rats treated with *P. cupana* extract were lower than those measured in the rats that received the vehicle of the extract

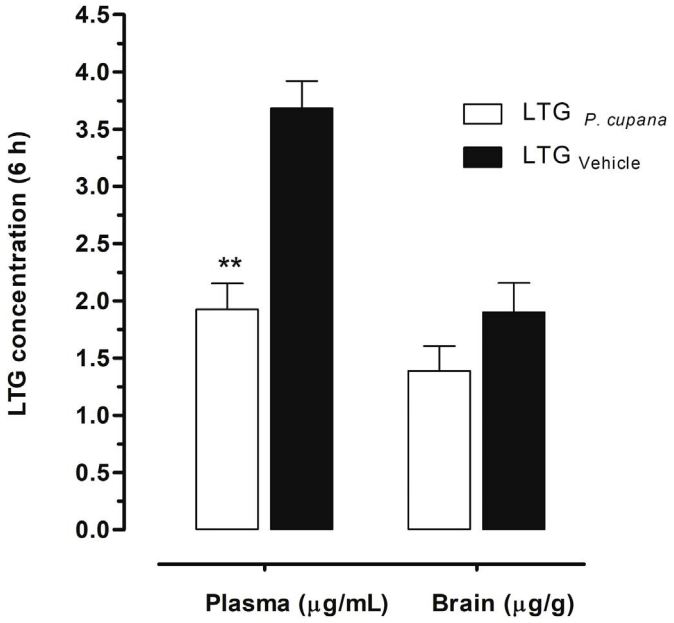

Fig. 3. Mean plasma and brain tissue concentrations of lamotrigine (LTG), obtained at 6 h post-dose, from rats co-administrated with a single-dose of *Paullinia cupana* extract (821 mg/kg, p.o.) or vehicle (i.e. water) and LTG (10 mg/kg, p.o.) by oral gavage. Data are presented as the mean values ± standard error of the mean (SEM) of five determinations ($n = 5$). **$p < 0.005$ compared to control (vehicle).

(i.e. water), being 79.2 ± 2.1 mg/dL and 94.5 ± 5.5 mg/dL, respectively. Regarding the total cholesterol levels, the values obtained were very similar in both experimental and control groups, with mean concentrations of 156.2 ± 1.4 mg/dL and 158.7 ± 2.1 mg/dL, respectively.

3.5. Effects of repeated-dose administration of P. cupana extract on body weight

The data regarding body weight of rats treated with *P. cupana* extract (821 mg/kg/day, p.o) or vehicle during 14 consecutive days are shown in Fig. 5. The rats of both control and experimental groups had a

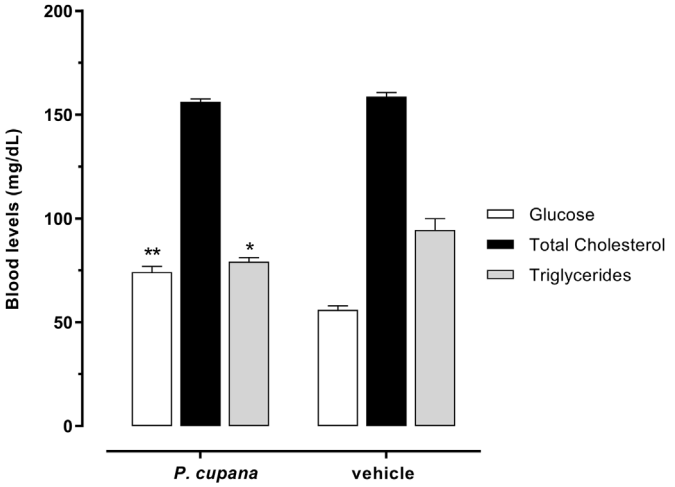

Fig. 4. Effects of *Paullinia cupana* extract on biochemical parameters (blood glucose, total cholesterol and triglycerides) after a 14-day treatment period with *Paullinia cupana* extract (821 mg/kg/day, p.o.) or vehicle (water) by oral gavage. Data are presented as the mean values ± standard error of the mean (SEM) of six determinations ($n = 6$). $^{*}p < 0.05$ and $^{**}p < 0.005$ compared to control (vehicle).

similar body weight at the beginning of the study (day 1), with mean values of 250.7 ± 6.0 g and 243.3 ± 5.4 g, respectively. From the results obtained, a statistically significant increase in the body weight of rats was observed between day 1 and day 14 in both experimental (*P. cupana*) and control (vehicle) groups ($p < 0.005$). When comparing the body weight gains of the rats during the period of the study, a trend towards a lower weight increase was observed in the rats that received *P. cupana* extract, however, such difference was not found to be statistically significant ($p = 0.06$).

4. Discussion

The goal of AED therapy is to control seizures and improve the patient's quality of life. Drug-related problems, including those resulting from interactions between herbal substances and AEDs can influence the efficacy, safety and adherence to AED therapy. Also of concern is the fact that many patients believe in the safety of herbal medicines and therefore do not report its use to the physician. In a study involving 92 patients with epilepsy, it was found that 24% were using complementary and alternative therapies, of which 41% were using herbs and supplements and, in most cases, it was not of the doctor's knowledge (Peebles et al., 2000). In a more recent study conducted by Eyal et al. (2014), in adult patients with epilepsy, the results showed that 48% of them took dietary supplements simultaneously with AEDs and patient awareness for potential drug interactions involving AEDs was very limited.

The pharmacokinetic studies herein reported were designed to assess the potential of interaction between *P. cupana* extract and LTG *in vivo* in Wistar rats. Considering that no interaction has been previously reported among these components, our starting point for a preliminary preclinical risk assessment was to evaluate the effects of *P. cupana* extract on the LTG bioavailability after their simultaneous administration, aiming at investigating a possible interference of *P. cupana* extract on the gastrointestinal absorption of LTG. In these experimental conditions, the obtained pharmacokinetic results clearly evidenced a decrease in absorption rate of LTG from the gastrointestinal tract of rats (as denoted by C_{max} and $AUC_{0\text{-}24}$), even though no important differences were detected in the extent of total systemic drug exposure (as assessed by $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$). These findings support that *P. cupana* extract, or some of its constituents, interact in some way with LTG in the gastrointestinal tract of rats, delaying the drug absorption. These data converge with results previously reported by our research group in a similar study involving *P. cupana* extract and amiodarone, in which a significant reduction in the peak plasma concentration (73.2%) and in the extent of systemic exposure (57.8%) to amiodarone were found (Rodrigues et al., 2012). The simultaneous co-administration of a *Fucus vesiculosus* extract and amiodarone also resulted in a significant decrease (55.4%) of the peak plasma concentration and in a reduction of approximately 30% in the extent of systemic exposure to amiodarone (Rodrigues et al., 2013). Moreover, similar results were found *in vivo* after oral pretreatment of rats with green tea extract (175 mg/kg/day) for 4 days followed by a single-dose administration of clozapine 20 mg/kg, which resulted in a significant decrease of C_{max} and $AUC_{0\text{-}\infty}$. The authors suggested that green tea extract delayed the gastric emptying of clozapine, reducing the rate and amount of clozapine absorbed (Jang

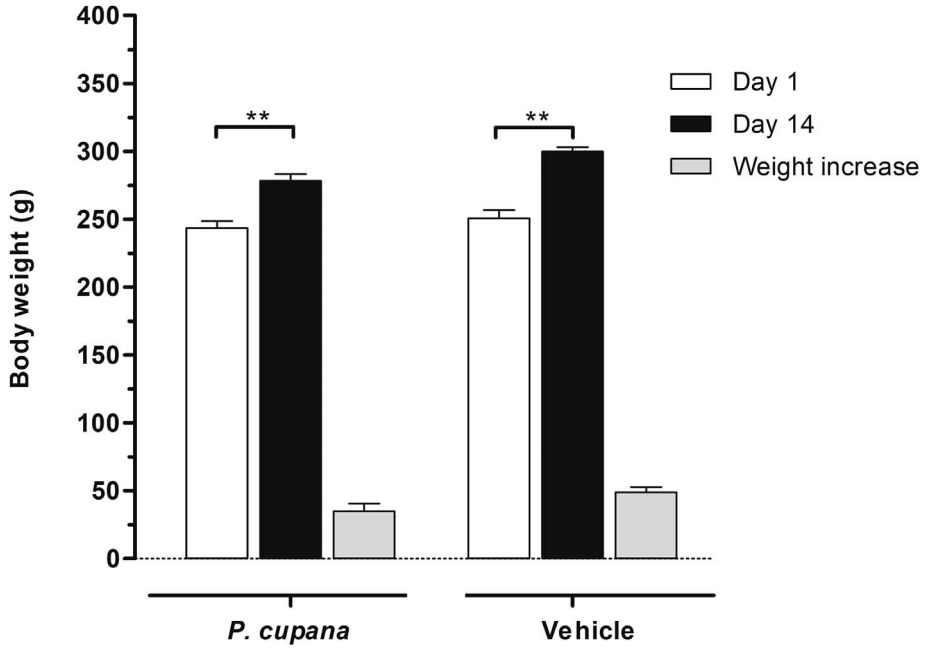

Fig. 5. Effects of *Paullinia cupana* extract on the body weight of rats after a 14-day treatment period with *Paullinia cupana* extract (821 mg/kg/day, p.o.) or vehicle (i.e. water) by oral gavage. Data are presented as the mean values ± standard error of the mean (SEM) of six determinations ($n = 6$). $^{**}p < 0.005$, day 1 *versus* day 14.

et al., 2005).

Moreover, having in mind the central role that induction of enzymes and transporters plays on drug-drug and herb-drug interactions, and knowing that the induction mechanisms are time-dependent, a second study was delineated to evaluate the effects of the repeated administration of *P. cupana* extract on the pharmacokinetics of LTG. The *P. cupana* extract pretreatment for 14 days resulted in a slightly higher systemic exposure to LTG, however, no important differences were found in comparison with the control group. These results suggest that *P. cupana* extract can interact with LTG disposition, but the similarity observed in the extent of systemic drug exposure in the rats of both experimental and control groups excludes the impact of *P. cupana*-induced metabolism on the bioavailability of LTG. Therefore, by combining the results of the two pharmacokinetic studies, it can be inferred that the herb-drug interaction between *P. cupana* extract and LTG found in the co-administration study occurred mainly at the absorption level, being unlikely the involvement of metabolism-based mechanisms. Indeed, as LTG has an oral bioavailability of approximately 100% and its absorption is not affected by food (Garnett, 1997), and taking also into account the available evidence that LTG permeates through biological mucosa mainly via the non-storable transcellular passive diffusion (Mashru et al., 2005), there is a negligible probability of this reported herb-drug interaction being the result of competition mechanisms by the same carrier. Thus, considering all the results presented herein, it is plausible to hypothesize that a physical-chemical interaction occurred between *P. cupana* extract, or its constituents, and LTG in the gastrointestinal tract of rats, which may explain the decrease in the rate of systemic exposure to LTG after its simultaneous co-administration with *P. cupana* extract. Thus, we presume that the effect of *P. cupana* extract could be related to the adsorption of lamotrigine in an identical manner to the effect caused by charcoal on lamotrigine (Keränen et al., 2011). Nevertheless, further studies are needed to better understand the mechanism associated with this herb-drug interaction (*P. cupana* extract/LTG), which is herein reported for the first time. Although there are pharmacokinetic differences between species, the effective plasma levels of AEDs are usually quite similar among rodents and humans (Castel-Branco et al., 2005; Loscher, 2011); hence, the clinical relevance of this interaction must be further investigated in order to understand the therapeutic impact of a lower systemic incorporation rate of LTG.

The repeated administration of *P. cupana* extract for 14 days had effects on glucose and triglyceride levels, increasing the glycaemia and reducing the blood levels of triglycerides. Another study identified similar results in rats treated with *P. cupana* extract, showing an increase in the glycaemia and a decrease in blood triglyceride levels in the experimental groups (Antonelli-Ushirobira et al., 2010). The reduction in blood triglyceride levels after *P. cupana* intake was also observed in human studies (Krewer et al., 2011; Portella et al., 2013; Suleiman et al., 2016). Indeed, caffeine, a methylxanthine abundantly present in *P. cupana* extract, has already been related to inhibitory effects on pancreatic lipase, a key enzyme in the dietary absorption of triacylglycerols (Yun, 2010). Portella et al. (2013) also demonstrated that *P. cupana* extract has peroxyl radical scavenger activity and inhibits lipid peroxidation, which may explain the impact of the extract on the lipid metabolism. Although no statistically significant difference was observed in cholesterol levels, methylxanthines have been related to the control of the transcription of genes for 3-hydroxy-3-methylglutaryl coenzyme A reductase and low density lipoprotein receptor (Ruchel et al., 2017). Likewise, the 14-day treatment period with *P. cupana* extract did not have a strong effect on the body weight of rats; however, according to the available data, there appears to be a tendency for a slower weight gain in the rats of the experimental (*P. cupana*) group. Antonelli-Ushirobira et al. (2010) also found no statistically significant differences in rats' body weight after 14 days of administration of *P. cupana* extract; nevertheless, when the animals were treated for a longer period of time (90 days) a slower increase in the body weight gain was observed. These effects of *P. cupana* extract intake on the body weight of rats and in the measured biochemical parameters reinforce the potential benefits of this extract in weight management and in lipid metabolism.

5. Conclusion

This work is the first report documenting the occurrence of a herb-drug interaction between *P. cupana* and LTG after their simultaneous co-administration, which led to a significant reduction in the rate and extent of systemic exposure to LTG. On the other hand, the repeat pretreatment with *P. cupana* did not have a significant impact on LTG concentrations when the drug was administered 24 h after the last administration of the extract. Thus, bearing in mind the effects of *P. cupana* extract on the systemic absorption of LTG, it is prudent to advise patients on therapy with LTG to avoid the simultaneous ingestion of *P. cupana*-containing products, thus minimizing the risks of occurrence of interaction. However, if the treatment with *P. cupana*-containing products and LTG is required at the same time in a patient, then they should be administered separately on the day (one in the morning and the other in the evening).

Conflicts of interest

The authors have declared no conflict of interests.

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